

WAGENINGENUR

P.O. Box PO Box 619 | 6700AP Wageningen | The Netherlands

To whom it may concern:

Regarding the submitted Ph.D Thesis "The complicated evolution of methionine adenosyltransferase in euglenids and eukaryotes in general", as a referee I consider that the defendant present her relevant work accordingly to the standards required and I recommend it for a Ph.D defense process.

The thesis is focused on the role of the enzyme methionine adenosyltransferase (MAT) and the mysterious duality with a different type of MAT (MATX) present sparsely distributed among some eukaryotic groups (euglenids, diatoms, haptophytes, dinoflagellates), in a similar fashion as EF1/EFL. The detailed introduction provides the context and phylogenetic framework in which this duality certainly could be relevant to understand eukaryotic evolution using its presence/absence as an independent phylogenetic marker. The quality of this Thesis is easily assessed by the impact factor of the publications attached (MBE and BMC Evolutionary biology) in which Jana is the first author. The technical and scientific goals are remarkably challenging (e.g. the silencing MAT in Trypanosoma with 9 endogenous copies). These chapters collect the efforts to understand the mechanisms of replacement between these two forms and extend the phylogenetic sampling on euglenids in order to compare the timing of the plastid acquisition and the MAT/MATX duality. A third paper (PLOS One) analyze the chloroplast genomic organization for Eutreptiella compared to Euglena adding an outgroup to understand plastid gene content evolution in this phylogenetic group. Finally, a quite short conclusion wraps up the arguments regarding the possible nature and evolution of MAT and MATX.

The main results shown in this Thesis do not provide a black and white scenario for the evolution of MAT/MATX and two main hypothesis are used to explain the observed occurrence: deep paralogy and horizontal gene transfer. I found that the conclusions are quite categorical based on the existing evidence and in my opinion other scenarios should have been explored more thoroughly during the discussion. The candidate is aware of extensive previous literature and it is clear how this work represents a step forward in the field. However, it is difficult to assess the candidate's reflection on the research as the conclusions are short and not too elaborated. Equally, the quality of written presentation is good base on the Introduction and Conclusions but it not possible to evaluate candidate's contribution on writing the papers.

In overall, this thesis represents an important step forward in knowing the evolution of MAT/MATX in eukaryotes on plastid evolution. The subject of this thesis is quite close to my previous research and I found very stimulating the efforts presented in this Thesis. I encourage keep investigating in the proposed lines of research as new insights on the evolution in these phylogenetic groups could be unravelled using this unique markers.

## Bioscience

## batte 16th January, 2015

sumecr Reference Ph.D. Thesis Mgr. Jana Szabová. "The complicated evolution of methionine adenosyltransferase in euglenids and eukaryotes in general

POSTAL ADDRESS P.O. Box PO Box 619 6700AP Wageningen The Netherlands

VISITORS' ADDRESS Wageningen Campus Building 107 Droevendaalsesteeg 1 6708 PB Wageningen

INTERNET WWW.wageningenUR.nl/pri

HANDLED BY Gabino F. Sanchez Perez

TELEPHONE +31 317481472

MAIL Gabino.sanchezperez@wur.nl

Wageningen UR (Wageningen University and various research institutes) is specialised in the domain of healthy food and living environment. Sincerely yours,

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Gabino F. Sanchez Perez Cluster Leader 'Applied Bioinformatics' and Senior Researcher Bioinformatics Department at Wageningen UR

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